THE USE OF DESSICATED TISSUE IN THE PREPARATION OF MEDIA FOR THE CULTIVATION OF FASTIDIOUS MICRO-ORGANISMS

BY

BERNARD ALBERT STEINBERG

A.B., New York University, 1947
M.S., University of Illinois, 1948

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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Introduction

Since the introduction of liquifiable solid media to the field of micro-biology there have been a great many forward steps made. It is very likely that many of these advances could not have been possible without agar or gelatin media. However, pathogenic or parasitic organisms require more specialized media than those used for the common bacteria. Blood, ascitic fluid or other tissues may be required for the growth of these fastidious micro-organisms. The addition of these enrichments to media make the problem of supply and storage a serious one. Many formulas call for fresh horse blood or ascitic fluid, thus requiring a constant supply and making prolonged storage impossible.

A process was available by which animal or plant tissues are dessicated and defatted without an apparent appreciable loss of their physiological activity. The tissues thus produced are stable and non-hygroscopic. They are able to be kept in the laboratory without any evident deterioration. If these tissues could be included in a medium in such form that they are available to the bacteria, then a great many problems of enrichment could be solved.

Method

Five genera of organisms were used; these were *Neisseria, Leptospira, Mycobacterium, Brucella* and *Hemophilus.* In all cases those tissues were used which are usually the site of infection in the animal body. However, some tissues selected for inclusion in the media were those which are commonly used to enrich standard media, e.g., egg and potato for *Myco-*

bacterium and blood for inclusion in blood agar on which an attempt was made to cultivate *Neisseria* and *Hemophilus.*

Powder, made from freshly killed animal tissue, was sterilized by exposure to ethylene oxide gas for a period of three hours. The tissue was then either suspended in sterile water or was digested with pancreas powder at 4°C for 24 hours in sterile buffered (pH 7.4) water. In either case it was then added to the melted basal medium. Slant tubes or poured plates were made from these media. At first undigested tissue was used, but results showed that the bacteria were apparently unable to use the enrichment thus prepared and, therefore, the tissues used later were all treated with the pancreas powder.

The cultures used in this investigation were as follows: *Leptospira icterohemorrhagiae* No. 917 and *Leptospira canicola* No. 1126. These were obtained from the Hooper Foundation of the University of California. *Brucella abortus* No. 4315, *Brucella suis* No. 9843 and *Brucella melitensis* No. 10,001 were procured from the American Type Culture Collection in Washington, D.C. The A.T.C.C. also supplied *Neisseria gonorrheae* No. 9793, *Hemophilus pertussis* No. 9832, *Hemophilus influenzae* type a No. 9006, *Hemophilus influenzae* type b No. 9334 and the H37Rv strain of *Mycobacterium tuberculosis,* A.T.C.C. No. 8236. An extremely fastidious strain of Brucella abortus (designated No.1) was obtained from the stock culture collection of this department.

The organisms were streaked on the surfaces of the experimental media and incubated at optimum conditions. Purity of the cultures was checked periodically by common staining procedures or dark-field examination in the case of *Leptospira.* ..

Results

The organisms of the *Leptospira* group grew well in a broth made up of sterile buffered water plus digested blood added to a final concentration of 8, 10, and 12%. Good growth was also obtained in sterile water to which digested liver or spleen had been added. These latter two were then used in

various combinations with each other and with blood. All of the combinations gave good results.

Mycobacterium tuberculosis grew well on a simple medium devised by the author, if the medium was enriched with certain tissues. A combination of egg and potato, if digested together with pancreas powder,allowedfor luxurious growth of the H37 strain in 3 days.

The composition of the basal is as follows:

Asparagine	0.2%
Glycerine	4.0%
Agar	1.5 - 2.0%
Distilled water	to one liter

This basal enriched with digested blood also supported growth but the growth was not luxurious nor were the results as consistent as on the potato-egg medium.

The addition of graduated amounts of ethylene dichloride soluble fraction of egg to the basal medium proved to be inhibitory to the organisms. The medium also failed to support growth if the glycerine was omitted from the formula. Similarly, if asparagine was not included there was no growth.

The members of the *Brucella* group obtained from the American Type Culture Collection all gave similar results. The strain of *Brucella abortus* designated No. 1 differed markedly in its requirements. This latter strain would not grow on Bacto-trytose agar unless blood was added and an atmosphere of 10% carbon dioxide was also essential.

Using B-B-L trypticase soy agar as a basal medium, enrichments were made with bovine and human placenta (digested). All strains including No. 1 showed good growth on this medium. Bacto-Nutrient agar and a medium made up of Wilson's peptone as a base were also usd. These allowed for the growth of the A.T.C.C. strains but No. 1 would not grow.

Hemophilus pertussis grew well on Bordet-Gengou agar base enriched with blood and potato, blood or potato, and a

combination of digested blood, potato, and *Staphylococcus aureus.* The latter allowed for luxuriant growth while the others only worked well in an atmosphere of 10% carbon dioxide. In no case was tissue used without being first digested.

Hemophilus influenzae and *Neisseria gonorrheae* were unable to be cultivated on any of the many tissues tried.

Summary

Members of the *Leptospira* and *Brucella* groups, *Mycobacterium tuberculosis* and *Hemophilus pertussis* were able to be cultivated on media containing digested tissue as an enrichment. A simple basal medium was devised for the growth of *Mycobacterium tuberculosis.*

The tissues used, produced by an azeotropic process, U.S. Patent numbers 250312 and 250313, were most useful if they were first digested before inclusion in the medium. It seems evident that the bacteria cannot utilize whole protein thus necessitating digestion. This may indicate that parasitic organisms utilize products of tissue metabolism in the animal body rather than actually destroy intact tissue.

VITA

The author was born in New York City on October 2, 1924. He attended the elementary and secondary schools in New York City and was graduated from De Witt Clinton High School in January, 1941. He received the Bachelor of Arts degree from the Heights division of New York University in May 1947. The Master of Science degree in Bacteriology was conferred on the author in August, 1948 by the University of Illinois.

The author spent 29 months in the United States Army and served in the European Theater of Operations. He was appointed a fellow in Bacteriology in February, 1949 and has held a fellowship since that time. He also assisted in a laboratory course in beginning bacteriology.

He is an active member of Sigma Xi, Phi Sigma and is also a member of the Society of American Bacteriologists.